

FIGURE 1 A  
NUCLEOTIDE SEQUENCE

>protease D-G (SEQ.ID.NO.:1)

CAACTTCACTTGTAGGGCTTTAATCAAGCTGCCAAAGTCCCCAATCACTCCTGGA  
ATACACAGAGAGAGGCAGCAGCTGCTCAGCGGACAAGGATGCTGGCGTGAGGGACCAA  
GGCCTGCCCTGCACTCGGGCCTCCTCCAGCCAGTGCTGACCAGGGACTTCTGACCTGCTG  
GCCAGCCAGGACCTGTGTGGGGAGGCCCTCCTGCTGCCTGGGGTACAATCTCAGCTCC  
AGGCTACAGGGAGACCGGGAGGGATCACAGAGCCAGCATGGATCCTGACAGTGATCAACCT  
CTGAACAGCCTCGATGTCAAACCCCTGCGCAAACCCGTATCCCCATGGAGACCTTCAGA  
AAGGTGGGGATCCCCATCATCATAGCACTACTGAGCCTGGCAGTATCATCATTGTGGTT  
GTCCTCATCAAGGTGATTCTGGATAAAATACTACTTCCCTGCGGGCAGCCTCTCCACTTC  
ATCCCAGGAAAGCAGCTGTGTGACGGAGAGCTGGACTGTCCCTGGGGAGGACGAGGAG  
CACTGTGTCAAGAGCTTCCCAGGGCCTGCAGTGGCAGTCCGCCTCTCCAAGGACCGA  
TCCACACTGCAGGTGCTGGACTCGGCCACAGGAACGGTCTCTGCCTGTTCGACAAC  
TTCACAGAACGCTCTCGCTGAGACAGCCTGTAGGCAGATGGCTACAGCAGCAAACCCACT  
TTCAGAGCTGTGGAGATTGGCCAGACCAAGGATCTGGATGTTGAAATCACAGAAAAC  
AGCCAGGAGCTCGCATGCGGAACCTCAAGTGGCCCTGTCTCTCAGGCTCCCTGGCTCC  
CTGCACTGTCTGCCTGTGGAAAGAGCCTGAAAGACCCCCCGTGTGGTGGGGAGGAG  
GCCTCTGTGGATTCTGGCCTGGCAGGTCAAGTCAGTACGACAAACAGCACGTC  
GGAGGGAGCATCCTGGACCCCCACTGGTCCTCACGGCAGCCCAGTCTCAGGAAACAT  
ACCGATGTGTTCAACTGGAAGGTGCGGGCAGGCTCAGACAAACTGGCAGCTCCATCC  
CTGGCTGTGCCAAGATCATCATCATTGAATTCAACCCATGTACCCAAAGACAATGAC  
ATCGCCCTCATGAAGCTGCAGTCCCACTCACCTTCTCAGGCACAGTCAGGCCATCTGT  
CTGCCCTTCTTGATGAGGAGCTCACTCCAGCCACCCCCACTCTGGATCATTGGATGGGC  
TTTACGAAGCAGAATGGAGGGAAAGATGTCTGACATACTGCTGCAGGCAGTCAGTCCAGGTC  
ATTGACAGCACACGGTCAATGCAGACGATGCGTACCTGGGGAAAGTCACCGAGAAGATG  
ATGTGTGCAGGCATCCCGGAAGGGGTGTGGACACCTGCCAGGGTACAGTGGTGGGCC  
CTGATGTACCAATCTGACCAGTGGCATGTGGTGGCATCGTTAGCTGGGTATGGCTGC  
GGGGGCCGAGCACCCCCAGGGTATACACCAAGGTCTCAGCCTATCTCAACTGGATCTAC  
AATGTCTGGAAGGCTGAGCTGTAATGCTGCTGCCCTTGCAAGTGTGGAGCCGCTTCC  
TTCCTGCCCTGCCACCTGGGATCCCCAAAGTCAGACACAGAGCAAGAGTCCCTGG  
GTACACCCCTCTGCCACAGCCTCAGCATTCTGGAGCAGCAAAGGCCCTCAATTCTA  
TAAGAGACCCCTCGCAGCCCAGAGGCGCCAGAGGAAGTCAGCAGCCTAGCTCGGCCACA  
CTTGGTGCTCCAGCATCCAGGGAGAGACACAGCCACTGAACAAAGGTCTCAGGGTAT  
TGCTAAGCCAAGAAGGAACCTTCCCACACTACTGAATGGAAGCAGGCTGTCTGTAAAAG  
CCCAGATCACTGTGGCTGGAGAGGAGAAGGAAAGGGTCTGCGCCAGCCTGTCCGTCTT  
CACCCATCCCCAAGCCTACTAGAGCAAGAAACCAGTTGTAATATAAAATGCACTGCCTAC  
TGTGGTATGACTACCCTACTGTTGTCATTGTTATTACAGCTATGCCACTATTA  
TTAAAGAGCTGTGTAACATCA

**FIGURE 1 B**  
**AA SEQUENCE**

>protease D-G (SEQ.ID.NO.:2)

MDPDSDQPLNSLDVKPLRKPRIPMETFRKV**GIPIIALLSLASIIIVVVLIK**VILDKYYF  
LCGQPLHFI**PRKQLCDGEELDCPLGEDEEHCVKSFP**EGPAVAVRLSKDRSTLQVLDSATGN  
WFSACFDNFTEALAE**TACRQM**GYSSKPTFRAVEIGPDQDLDVVEITENSQELMRMRNSSGP  
CLSGSLVSLH**CLACGKSLKT**PRVGEEASVDSWPWQVSIQYDKQHVC~~GG~~SILDPHWVLT  
AAHCFRKHTDVFNWKVRAGSDKLGSFPSLAVAKIIII**E**FNPMPYPKDNDIALMKLQFPLTF  
SGTVRPICL~~PFF~~DEELTPATPLWIIGWGFTKQNGGKMSDILLQASVQVIDSTRCNADDAY  
QGEVTEKMMCAGIPEGGVDTQCQGDGGPLMYQSDQWHVVGIVSWG~~Y~~GC~~GG~~PSTPGVYTKV  
SAYLNWIYNVWKAEL

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FIGURE 2  
PHYLOGENETIC TREE

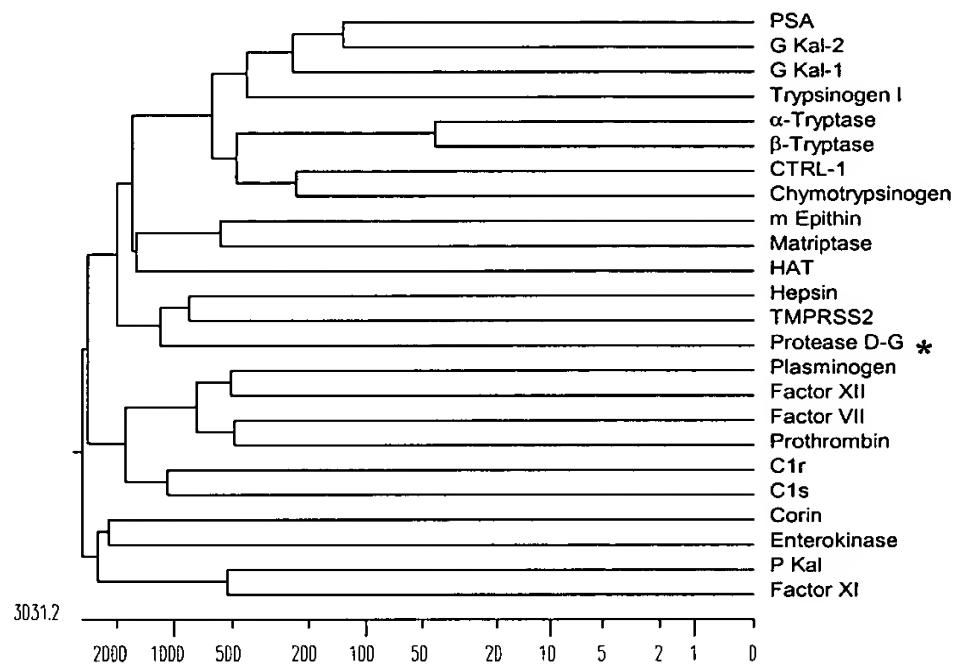
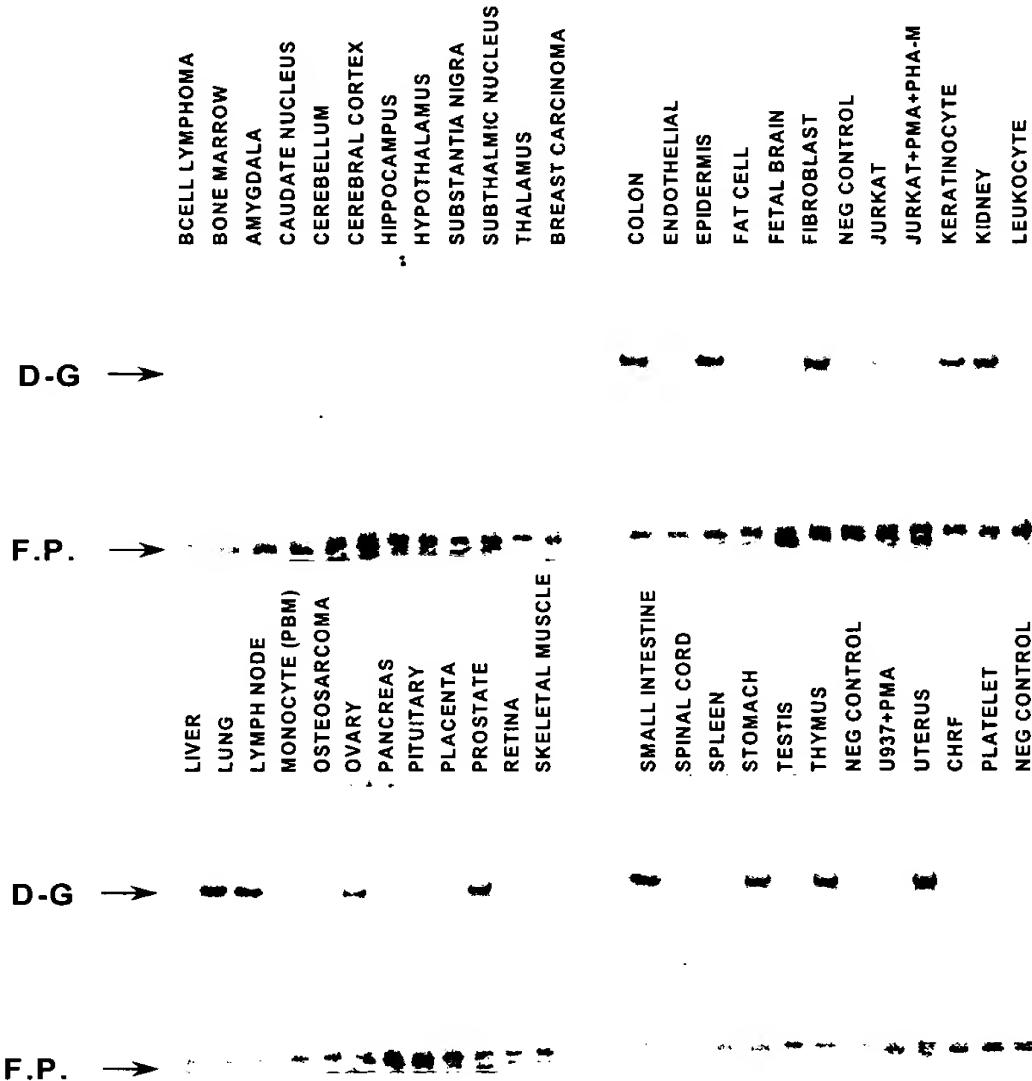


FIGURE 3  
TISSUE DISTRIBUTION

Protease D-G mRNA Tissue Distribution



**FIGURE 4 -A**  
**CONSTRUCT NUCLEOTIDE SEQUENCE**

>PFEK-D-G-HIS ERI-HCII (SEQ.ID.NO.:8)

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GAATTCAACCACCATGGACAGCAAAGGTTCGCAGAAATCCCGCTGCTCCTGCTGCTG  
GTGGTGTCAAATCTACTCTTGTGCCAGGGTGTGGCTCCGACTACAAGGACGACGAC  
GTGGACCGCGCCGCTCTGCTGCCCTTGTGATGATGACAAGATCGTGGGGCTAT  
GCTCTAGATGTGGATTCTTGGCCTTGGCAGGTCACTGGGCACGGCAGCCCAGTC  
TGTGGAGGGAGCATTGGACCCCCACTGGGCCTCACGGCAGCCCAGTCAGGAAA  
CATACCGATGTGTTCAACTGGAAGGTGCAGGCTCAGACAAACTGGGCAGCTTCCA  
TCCCTGGCTGTGGCCAAGATCATCATCATTGAATTCAACCCATGTACCCAAAGACAAT  
GACATCGCCCTCATGAAGCTGCAGTCCCCTCACTTTCTCAGGCACAGTCAGGCCATC  
TGTCTGCCCTTCTTGATGAGGAGCTCACTCCAGCCACCCACTCTGGATCATTGGATGG  
GGCTTACGAAGCAGAATGGAGGAAGATGTCTGACATACTGCTGCAGGCGTCAGTCCAG  
GTCATTGACAGCACACGGTCAATGCAGACGATGCGTACCTGGGGAAAGTCACCGAGAAG  
ATGATGTGTCAGGCATCCCGAACGGGGTGTGGACACCTGCCAGGGTACAGTGGTGGG  
CCCCTGATGTACCAATCTGACCATGGCATGTGGCATGTGGTGGCATCGTTAGCTGGGCTATGGC  
TGCGGGGGCCGAGCACCCAGGGTATACACCAAGGTCTCAGCCTATCTCAACTGGATC  
TACAATGTCTGGAAGGCTGAGCTGTCTAGACATCACCACCATCACTAGCGGCCGTT  
CCCTTAGTGAGGGTTAATGCTCGAGCAGACATGATAAGATACTTGATGAGTTGGAC  
AAACCACAACCTAGAATGCAGTGAAAAAAATGCTTATTGTGAAATTGTGATGCTATTG  
CTTATTGTAAACCATTATAAGCTGCAATAAACAGTTAGCTTGTGAGAAGTACTAGAG  
GATCATAATCAGCCATACCACATTGTAGAGGTTACTTGCTTAAAAACCTCCACAC  
CCTCCCCCTGAACCTGAAACATAAAATGAATGCAATTGTTGTTAAC
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**FUSION PROTEIN**

>PFEK-D-G-HIS (SEQ.ID.NO.:9)

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MDSKGSSQKSRLLLLLVVSNLLLCQGVVSDYKDDDDVDAAALAAPFDDDKIVGGYALDVDS  
WPWQVSIQYDKQHVCAGSILDPHWVLTAAHCFRKHTDVFNWVKRAGSDKLGSFPSLAVAKII  
IIEFPMPYPKDNIDIALMKLQFPLTFSGTVRPICLPPFDEELTPATPLWIIGWGFTQNGGKM  
SDILLQASVQVIDSTRCNADDAYQGEVTEKMMCAGIPEGGVDTQCQGDGGPLMYQSDQWHVV  
GIVSWGYZCAGPSTPGVYTKVSAYLNWIYNVWKAELSRHHHHHH
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FIGURE 4-B

GAATTCAACCATGGACAGCAAAGGTTCTCGCAGAAATCCCGCTGCTCTGCTGGTGTCAAATCTACTCTTGCCAGGGTGTGGCTCCG  
CTTAAGTGGTGTACCTGCTTTCAAGCAGCGTCTTAGGGCGAGGACGACGACCACAGTTAGATGAGAACACGGTCCCACACCAGAGGC + 100

M D S K G S S O K S R L L L L V V S N L L C O G V V S  
Prolactin Signal Sequence

Not I Xba I  
ACTACAAGGACGACGACGTGGACGCCGCTTGTGCTGCCCTTGTGATGATGACAAGATCGTTGGGGCTATGCTCTAGATGTGGATTCTG + 200  
TGATGTTCTGCTGCTGCTGACCTGCGCCCGAGAACGACGGGGAACTACTACTACTGTTCTAGAACCCCCGATACGAGATCTAACCTAAGAAC  
D Y K D D D D V D A A A L A A P F D D D D K I V G G Y A L D V D S W  
FLAG BK

GCCTTGGCAGGTCAAGCATCCAGTACGACAAACAGCACGCTGTGGAGGGAGCATCTGGACCCCCACTGGTCTTCACGGCAGCCCACGTCTCAGGAAA + 300  
CGGAACCGTCCAGTCGTAGGTCACTGCTGTTGCTGCAAGACACTCCCTCGTAGGACCTGGGGTAGCCAGGAGTGGCTCGGGTGACGAAGTCTTT  
P W Q V S I O Y D K O H V C G G S I L D P H W V L T A A H C F R K  
Protease D-G Catalytic Domain

CATAACCGATGTTCAACTGGAAGGTGCGGGCAGGCTCACGACAAACTGGCGACTTCCATCCCTGGCTGTGGCCAAGATCATCATATTGAATTCAACC + 400  
GTATGGCTACACAAGTTGACCTTCCACGCCGTCGAGTCTGTTGACCCGTCGAAGGGTAGGGACCGACACCGTTCTAGTAGTAACTTAAGTGG  
H T D V F N W K V R A G S D K L G S F P S L A V A K I I I E F N  
Protease D-G Catalytic Domain

CCATGTACCCAAAGACAATGACATGCCCTCATGAAGCTGCAAGTCCCACACTCACTTCTCAGGCACAGTCAGGCCATCTGTGCCCTTCTTGATGA + 500  
GGTACATGGGTTCTGTTACTGTAGCGGGAGTACTCGACGTCAAGGGTAGTGAAGAGTCCGTCAAGTCCGGTAGACAGACGGGAAGAACTACT  
P M Y P K D N D I A L M K L Q F P L T F S G T V R . P I C L P F F D E  
Protease D-G Catalytic Domain

GGAGCTCACTCCAGCCACCCCACTCTGGATCATTGGATGGGGTTTACGAAGCAGAACATGGAGGGAAAGATGTCTGACATACTGCTGCAGCGTCAGTCCAG + 600  
CCTCGAGTGAGGTGGTGGGGTAGACCTAGTAACCTACCCGAAATGCTCGTCTACCTCCCTACAGACTGTATGACGACGTCCCGAGTCAGGTC  
E L T P A T P L W I I G W G F T K Q N G G K M S D I L L O A S V D  
Protease D-G Catalytic Domain

GTCATTGACAGCACACGGTCAATGCAGACGATGCGTACCGAGGGAAAGTCACCGAGAACATGGAGGGATGATGTGTGCAGGCATCCGGAAAGGGGGTGTGGACACCT + 700  
CACTAAGTGTGTTGCCCCAGTCTACGCTCTACCGATGGCTCTTCACTACACACGTCCGTAGGGCTTCCACACCTGTGA  
V I D S T R C N A D D A Y O G E V T E K M M C A G I P E G G V D T  
Protease D-G Catalytic Domain

GCCAGGGTGACAGTGGGGCCCTGATGTAACCATCTGACCACTGGCATGTGGGGCATCGTTAGCTGGCTATGGCTGGGGCCGAGCACCC + 800  
CGGTCCCACTGTCAACCCCCGGGACTACATGGTAGACTGGTCACCGTACACCCCGTAGCAATCGACCGGATACCGACGCCCCCGCTGTGGG  
C Q G D S G G P L M Y Q S D O W H V V G I V S W G Y G C G G P S T P  
Protease D-G Catalytic Domain

Xba I Not I  
AGGAGTATAACCAAGGTCTAGCTATCTCAACTGGATCTACAATGTCTGGAAAGGCTGAGCTGTCTAGACATCACCATCACAGCGGCCGCTT + 900  
TCCTCATATGTGGTTCAGAGTGGATAGAGTTGACCTAGATGTTACAGACCTTCGACTCGACAGATCTGAGTGGTAGTGTGATCGCCGGCAA  
G V Y T K V S A Y L N W I Y N V W K A E L S R H H H H H H H .  
Protease D-G Catalytic Domain 6XHIS-TAG

CCCTTTAGTGAGGGTTAATGCTTCGAGCAGACATGATAAGATACTATTGATGAGTTGGACAAACACAACATAAGTCAGTGGAAAAATGCTTATTTG + 1000  
GGGAAATCACTCCAATTACGAAGCTCGTGTACTATTCTATGTAACACTACTCAAAACCTGTTGGTGTGATCTACCTCACTTTTACGAAATAAAC  
TGAAATTGATGCTATTGCTTATTTGTAACCAATTATAAGCTGCAATAAACACAAGTGTAGCTGTGAGGAGTACTAGAGGGATCATAATCAGCCATACCA + 1100  
ACTTTAACACTACGATAACGAAATAAACATTGGAATATTGACGTTATTGTTCAATCGAACAGCTTCACTGATCTCTAGTATTAGTCGGTATGGT  
CATTTGAGAGGTTTACTTGCTTAAAAAACCTCCCACACCTCCCCCTGAAACCTGAAACATAAAATGAATGCAATTGTTGTTAAC + 1189  
GTAAACATCTCAAATGAACGAAATTGAGGGTGGAGGGGACTTGGACTTTACTACGTTAACACAACAAATTG

**FIGURE 5**  
**PAGE - WESTERN BLOT**

**Protease D-G**

1 2 3 4 5  
- + - + EK

111.0  
73.0  
47.5  
33.9  
28.8

FIGURE 6

**Chromogenic Activity of Protease D-G**

